Male genital titillators and the intensity of postcopulatory sexual selection across bushcrickets

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Abstract

Animal genitalia are diverse and a growing body of evidence suggests that they evolve rapidly under post-copulatory sexual selection. This process is predicted to be more intense in polyandrous species, although there have been very few comparative studies of the relationship between the complexity of genital structures in males and measures of the degree of polyandry. In some bushcricket families, males possess sclerotised copulatory structures known as titillators, which are inserted into the female's genital chamber and moved rhythmically. Like other genital structures, bushcricket titillators are widely used as important taxonomic characters and show considerable variation across species in structure, shape and the extent to which they are spined. Here, we examine relationships between the presence/absence of titillators, titillator complexity and both mating frequency and the degree of polyandry in bushcrickets, using phylogenetic comparative analyses. Using published sources combined with original observations, data were obtained for the mean level of polyandry, the duration of the male and female sexual refractory periods and the level of complexity of titillators. To analyse data, we fitted phylogenetic generalised least squares models. No significant relationships were found between titillator presence or complexity and either the level of polyandry, duration of the male's sexual refractory period or the ratio of the female and male sexual refractory periods. The duration of the female's refractory period, however, was positively associated with titillator presence and negatively associated with titillator complexity. The data therefore partially support the hypothesis that post-copulatory sexual selection drives genital evolution in this taxon.

Genital structures are extremely diverse and are therefore often used as taxonomic characters to separate closely related species (Eberhard 1985, 2010). Genital traits appear to diverge more rapidly than non-genital traits (Rowe and Arnqvist 2012). The selective pressures that drive such rapid evolutionary change in genitalia have been the subject of considerable debate. These include selection for species isolation (the “Lock and Key” hypothesis, reviewed in Mikkola 2008), neutral evolution (Pleiotropy hypothesis) and sexual selection (reviewed in Eberhard 2010; Simmons 2014). The sexual selection hypothesis potentially involves three closely inter-related processes of post-copulatory sexual selection: sperm competition, sexually antagonistic co-evolution and cryptic female choice (Simmons 2014). Strong support for the role of sexual selection in genital evolution comes from comparative studies that have found a positive relationship between indices of the degree of polyandry (and hence the intensity of sexual selection) and the degree of evolutionary divergence or elaboration of genital structures in mammals (Ramm 2007; Orr and Brennan 2016) and insects (Arnqvist 1998, Rowe and Arnqvist 2012).

Bushcrickets (Orthoptera: Tettigoniidae) are an excellent model taxon in which to test hypotheses relating to the evolution of copulatory structures. They are a diverse and relatively well studied family of insects, which exhibit varying degrees of polyandry (Gwynne 2001; Vahed 2006, 2007). Two types of sclerotized copulatory structures occur in male bushcrickets: the cerci and titillators. The cerci act as genital claspers (Rentz 1972, Vahed et al. 2014). Comparative evidence suggests that sexual conflict over the duration of ejaculate transfer has affected the form and function of the cerci in bushcrickets (Vahed et al. 2014, Lehmann et al. 2016). Titillators (Figure 1) are concealed structures that are

inserted into the female's genital chamber prior to spermatophore transfer and are moved rhythmically with contractions of the male's abdomen and phallic complex (Wulff et al. 2015, 2017; Wulff & Lehmann 2016). The tips of the male's titillators contact the sensilla-rich, un-sclerotised, soft tissues of the opening of the female's genital chamber during copulation, prior to spermatophore transfer (Wulff et al. 2015, 2017). Like other genital structures, bushcricket titillators are widely used as important taxonomic characters (Chamorro-Rengifo and Lopes-Andrade 2014) and show considerable variation across species in structure, shape and the extent to which they are spined (Vahed et al. 2011). Comparative evidence indicates that the presence of titillators is associated with longer copulation durations (prior to spermatophore transfer) in bushcrickets (Vahed et al 2011), but the relationship between titillator complexity and polyandry has not previously been examined. In fact, we are not aware of any previous studies that have tested for a relationship across species between direct measures of the degree of polyandry and genital complexity in any animal taxon (for studies that have used in-direct measures of the degree of polyandry or used a binary "monandrous versus polyandrous" classification, see Ramm 2007; Arnqvist 1998; Rowe and Arnqvist 2012; Orr and Brennan 2016; Kuntner et al. 2016). Bushcrickets are one of the few animal groups in which data on the lifetime degree of polyandry are available for a range of species (Vahed 2006).

Here, we use phylogenetic comparative analyses to test the prediction that genital complexity in males will be positively related to the intensity of sexual selection in bushcrickets. We examine the relationship between titillator complexity and two related measures of the intensity of sexual selection: the lifetime degree of polyandry and the

relative potential reproductive rates (the reciprocal of the sexual refractory period) of males and females. Where the male's potential reproductive rate exceeds that of the female (i.e. where the operational sex ratio is male biased), males are assumed to be under stronger sexual selection (Clutton-Brock & Parker 1992, Ahnesjö et al. 2001: see also Kokko et al. 2014 for a critical review).

It should be noted that our study is not designed to distinguish between the different mechanisms of postcopulatory sexual selection (i.e. sperm competition, cryptic female choice and sexually antagonistic co-evolution). One mechanism of postcopulatory sexual selection, however, may be distinguishable because it potentially makes the opposite prediction: If complex genitalia cause damage to the female's reproductive tract (see Crudginton and Siva-Jothy 2000) and thereby delay the female from re-mating, then more elaborate genitalia could be associated with a longer "time out" from mating (and therefore a lower potential reproductive rate in females and a lower degree of polyandry, see Stockley 2002; Kuntner et al. 2016).

Methods

Data on the mean lifetime degree of polyandry were obtained for 29 species of tettigoniid bushcrickets from published sources including our own studies (Supplementary Table S1). For the majority of these species, data on polyandry were obtained from counts of spermatodoses within the spermatheca of tettigoniids sampled at the end of the season in the field (Gwynne 1984, Vahed 2006, Vahed et al. 2011, Gwynne & Lorch 2013, Kaňuch et al. 2015; Gao Yong, pers. comm.). Spermatodoses are capsules of sperm that are formed within the female's sperm storage organ (spermatheca) each time she mates (Vahed 2003, 2006, Parker et al. 2017). Microsatellite analysis of sperm from spermatodoses in the bushcricket *Pholidoptera griseoaptera*, which had mated under natural field conditions, revealed that in over 80% of females, each spermatodose within the spermatheca was from a different male (Parker et al. 2017). Re-mating between pairs in bushcrickets is unlikely due to the long sexual refractory periods of both males and females that occur after each mating, which are associated with the transfer of the large spermatophores that are typical of this family (Vahed 2007). Because of this, spermatodose counts can be used to estimate the degree of polyandry in bushcrickets (Gwynne 1984, Vahed 2006, Vahed et al. 2011, Gwynne & Lorch 2013, Kaňuch et al. 2015). Complementary data were obtained from assessments of caged populations (in one species, *Lluciapomaresius stalii*, Bateman 1998), or marked field populations (in six taxa, Heller and von Helversen 1991, McCartney 2010) and, in two species, molecular analysis of sperm in the spermatheca and offspring of field-mated females (Hockham et al. 2004, Simmons et al. 2007). Caged populations could yield an exaggerated degree of polyandry (females may not be able to evade the males as effectively as they would

131 in the field). It should be noted, however, that in the bushcricket species in this study which
132 was examine in caged populations, *L. stalii*, the males are unable to force the female to
133 copulate and the females play an active role in pair formation and in mounting the male to
134 initiate copulation (Bateman 1998). In addition, the degree of polyandry for this species was
135 at the lower end of the spectrum (mean of 2.1 mates), rather than high. Conversely, studies
136 of marked individuals in field populations could under-estimate the degree of polyandry
137 because matings could be missed. The large spermatophore, which remains attached to the
138 female for several hours after copulation, however, means that matings are unlikely to have
139 been overlooked in these studies (Heller and von Helversen 1991, McCartney 2010).
140
141 Data on refractory periods for males and females came from individuals assessed for the time
142 to re-mating in the laboratory (Supplementary Table S1). Individual pairs were assigned to
143 observation cages at a time of day corresponding to the peak activity period for that species
144 (see Vahed 2007 for further details). Data from two Australian species were from mating
145 observations in field cages (Lehmann 2007, Lehmann and Lehmann 2007). These refractory
146 periods correspond with 'time-outs from matings' and are therefore tightly linked with the
147 concept of potential reproductive rate (= 1/time-out, Clutton-Brock & Parker 1992, Ahnesjö
148 et al. 2001).
149
150 **Titillator structure**
151 Data on titillator morphology (Figure 1) were obtained from taxonomic sources, chiefly Harz
152 (1969) for the majority of European species (Supplementary Table S1). We developed a
153 ranked classification system to reflect titillator complexity, with emphasis on the complexity

(e.g. presence/absence of visible spines; clumping of spines at the tip; single or double pair of projections) of the apical part of the titillators (median projection) which makes contact with the female during copulation (Wulff et al. 2015, 2017) (see Figure 1, Table 1). Titillators were scored blind (by both the first and the last authors) for a previous study (Vahed et al. 2011), i.e. without knowledge of polyandry or duration of male or female refractory periods.

Analysis

We split "titillator complexity" into two components analysed in parallel: presence/absence of titillators, analysed as a binary variable (henceforth titillator presence), and complexity of titillators where present (henceforth titillator complexity), analysed as a continuous variable. This is because it would make little sense to treat species without titillators effectively as bearing "titillators of zero complexity".

All analyses were conducted in R 3.2.0 (R Core Team 2015). To account for non-independence due to phylogenetic relatedness, we used comparative-phylogenetic statistical methods that account for this non-independence by modelling errors in the model residuals as a function of the phylogenetic distance between species and the underlying model of how traits evolve along branches. We analysed continuous data (refractory periods) using phylogenetic generalized least squares models (PGLS; Grafen, 1989, Martins and Hansen, 1997) using the pgls() function in the *caper* package (Orme et al. 2013), simultaneously estimating Pagel's λ, i.e. how closely the tree structure can predict covariance among the model's residuals (Pagel 1999, Freckleton et al. 2002). For analysis of binary or Poisson data

(titillator presence/absence and polyandry, respectively) we fitted Generalised Estimating Equation (GEE) models (Paradis & Claude 2002), which are more appropriate for non-normally distributed data (Paradis 2006), using the compar.gee() function in the *ape* package (Paradis et al 2004). We used standard residual plots to assess model fit by eye.

We initially tested whether, within our dataset, polyandry was associated with the duration of the female or male refractory periods and whether female and male refractory periods were associated, as would be predicted (Vahed 2006, 2007). We first fitted a PGLS model of polyandry with female or male refractory period as a predictor variable, then a PGLS model with female refractory period as a response and male refractory period as a predictor. Data on polyandry were log-transformed before analysis to improve model fit. Then, for each analysis of titillator structure (binary GEE models of presence; continuous PGLS models of complexity) we fitted models against predictor variables including polyandry, female refractory period and male refractory period. We performed analyses of potential reproductive rate directly on male and female reproductive rate, as this improved model fit. We additionally ran analyses including the reciprocal of the ratio of the male and female refractory period as a predictor variable. This represents the relative reproductive rates of males and females (a measure of the operational sex ratio).

To avoid confounds due to variable measuring techniques, we re-ran all analyses excluding species where polyandry was assessed using molecular rather than field-based methods (*R. verticalis, E. ephippiger*) or where field rather than lab-based methods were used to assess

198 the male refractory period (*P. affinis, P. v. veluchianus*) or the female refractory period (*P.*
199 *affinis, P. v. veluchianus, P. v. minor*). See Supplementary Table S1 for details.
200
201 The phylogeny used for all analyses was based primarily on the molecular phylogeny by
202 Mugleston et al. (2013) supplemented by the molecular phylogenetic information derived
203 from barcoding of Central European species (Hawlitschek et al. 2016), combined with the
204 morphological phylogeny developed by Naskrecki (2000) (this phylogeny did not use
205 titillators as a character). For the sub-family Tettigoniinae, we used the morphological
206 phylogeny provided by Rentz and Coless (1990) (majority consensus tree of 50 equally short
207 cladograms) because many of the genera were neither included in Mugleston et al. (2013)
208 nor in Naskrecki (2000). For phylogenetic relationships within the genus *Anonconotus*
209 (Tettigoniinae), we used an unpublished molecular phylogeny based on mtDNA (R. Szabo,
210 G. Carron, K. Vahed & M. Ritchie). For the genus *Poecilimon* (Phaneropterinae), we used
211 the molecular phylogeny given in Ullrich et al. (2010), for the *Poecilimon propinquus*-group
212 the mtDNA tree of Lehmann (1998). Branch lengths were not available and so we ran all
213 analyses twice, arbitrarily scaling branches according to node depth, following Grafen
214 (1989), or setting all branch lengths to 1. We conducted our analyses on the datasets for which
215 relevant data were available (n=48). For analyses involving subsets of the full dataset, the
216 full tree was pruned to the appropriate set of taxa only after arbitrary branch lengths had been
217 assigned, in order to preserve the node depths in the full tree. The datasets supporting this
218 article have been uploaded as Supplementary Table S1.
219

Results

Our analyses returned similar results regardless of whether we excluded minority methods, or how we assigned branch lengths (arbitrarily to 1 or scaling according to node depth). Thus, we present only results using all available data regardless of the method used to obtain them, and using Grafen's (1989) node-depth scaling to assign arbitrary branch lengths to the tree.

Polyandry

There was a high variation in polyandry in bushcricket females, ranging from as low as a mean of 1.5 matings up to a mean of 25 to 28 matings per female in *Anonconotus* species (Figure 2). The data were largely biased towards species with relatively low polyandry, i.e. between 1.5 and 3.1 numbers of matings over the female lifespan. Polyandry was significantly negatively associated with the duration of the female refractory period (GEE, df=1, P=0.013, n=12) and the male refractory period (GEE, df=1, P=0.047, n=14). Female and male refractory periods were positively associated with each other ($F_{1,25}$=18.23, p<0.001, n=27); in this model Pagel's λ had wide confidence intervals (0 to 0.906), indicating low certainty in the estimate of phylogenetic signal.

In the full dataset, titillators were absent in 15 species and present in 32; titillator complexity in species with titillators was distributed as shown in Figure 2. Polyandry was related neither to titillator presence (PGLS: $F_{1, 27}$=0.0142, P=0.91, n=29; Figure 3a) nor to titillator complexity (PGLS, $F_{1, 20}$=2.56, P=0.13, n=22; Figure 3b) and vice versa: polyandry was a good predictor neither of titillator presence (GEE, df=1, p=0.98, n=29) nor complexity

(PGLS, $F_{1, 20}$=2.56, P=0.13, n=22). Estimates of Pagel's λ ranged from 0.94 to 1.00 [95% CIs ~0.7 – 1] for these models, indicating a high degree of phylogenetic signal, i.e. covariance in model residuals could be predicted by phylogenetic distance.

Male refractory period

The male refractory period can span over several days, the most extreme mean data coming from *Antaxius hispanicus* with 7 days, and *Poecilimon thessalicus* with 6 days. In contrast, several species are able to re-mate within an hour down to a few minutes. Titillator presence did not reliably depend on the male refractory period (PGLS: $F_{1, 30}$ = 0.51, P = 0.41, n=32, Figure 3c) but titillator complexity showed a marginal trend suggesting that species with longer male refractory periods have more complex titillators (PGLS: $F_{1,18}$=3.71, P=0.069, n=20, Figure 3d). The phylogenetic signal estimate had wide confidence intervals (λ~0.81 [CI 0.28, 0.96]). The male refractory period did not predict titillator presence (GEE: df=1, P=0.49, n=32) but again showed a marginally positive association with titillator complexity (PGLS: $F_{1, 18}$=3.66, P=0.072, n=20, λ=0.77[CI 0.33, 0.95]).

Female refractory period

The female refractory period in most species was typically longer than that of the male, ranging from 1.07 h to 19 days. Unlike the male refractory period, the female refractory period was positively associated with titillator presence (PGLS: $F_{1, 26}$=9.04, P=0.005, n=28; λ=0.62, Figure 3e) and negatively associated with titillator complexity (PGLS: $F_{1,13}$=18.052, n=15, P<0.001; λ=0, Figure 3f). Analysing the inverse relationships, the female refractory

period did not predict titillator presence (GEE, df=1, P=0.15, n=28) but was a good predictor of titillator complexity ($F_{1,13}$=18.052, P<0.001, n=15; λ=0).

Male vs female potential reproductive rates

The Operational Sex Ratio (measured as the reciprocal of the ratio of female and male refractory periods) was not associated with titillator presence (PGLS: $F_{1, 25}$=1.164, P=0.16, n=27, λ=0.85; Figure 3g) nor with titillator complexity (PGLS: $F_{1, 13}$=0.94, P=0.35, n=15; λ=0.60; Figure 3h). The inverse relationships were similarly nonsignificant, as the Operational Sex Ratio predicted neither titillator presence (GEE: df=1, P=0.73, n=27) nor complexity (PGLS: $F_{1, 13}$=1.00, P=0.34, n=15; λ=0.66).

Discussion

No significant relationships were found between titillator complexity and any of the degree of polyandry in bushcrickets, the duration of the sexual refractory period in males, or the relative potential reproductive rates of males and females. The duration of the female's sexual refractory period, however, was associated with both titillator presence and complexity. The results, therefore, provide partial support for the hypothesis that post-copulatory sexual selection is a driving force in titillator evolution. It should be noted, however, that while there was a high degree of variation between species in the degree of polyandry (ranging from a mean of 1.5 to a mean of 28 matings per lifetime), there were no entirely monandrous species in this data set. Any effect of post-copulatory sexual selection on genital evolution should be most detectable by comparing monandrous with polyandrous species (Arnqvist 1998). On the other hand, previous studies have found evidence for a positive relationship between indicators of the degree of polyandry, measured on a continuous scale, and the complexity or degree of development of the males' genitalia in both insects and mammals (Ramm 2007, Rowe and Arnqvist 2012, Orr and Brennan 2016).

The duration of the female's sexual refractory period was significantly longer in species in which titillators were present. On the face of it, this appears to be opposite to the prediction of the sexual selection hypothesis, which predicts that titillators should be more complex in species in which females are more polyandrous (and therefore have *shorter* sexual refractory periods, since there was a significant negative relationship between the duration of the female's sexual refractory period and the lifetime degree of polyandry in our present study).

298 The results are, however, consistent with the hypothesis that complex genitalia delay or deter
299 the female from remating, which can be driven by various mechanisms of post-copulatory
300 sexual selection. Stockley (2002), for example, found that in primates, relatively high penile
301 spinosity was associated with lower potential reproductive rates in females and interpreted
302 this in terms of internal damage caused to the female by the spines. Kuntner et al. (2016)
303 similarly found that in nephilid spiders, the male's genitalia were more complex in
304 polyandrous species than in monandrous species. Titillators in some bushcrickets have spines
305 (see Figure 1) that contact the soft, un-sclerotised lining of the female's bursa copulatrix
306 (Wulff et al. 2015, 2017). However, we found no evidence for any damage by the titillators
307 to the female's reproductive tract (Wulff et al. 2015, 2017; Wulff and Lehmann 2016), in
308 contrast to the action of penile spines in *Callosobruchus* beetles (Hotzy and Arnqvist 2009).
309 Comparative evidence suggests that genital damage selects for females to evolve a thicker
310 wall of the bursa copulatrix to minimise damage by the male's spines in seed beetles
311 (Coleoptera: Bruchidae) (Rönn et al. 2007). The thickness of the parts of the female contacted
312 by the male's genitalia was not measured in the present study. There is strong evidence from
313 other comparative studies of other insect taxa that co-evolution between male and female
314 genital structures does occur (Rönn et al. 2007, Yassin and Orgogozo 2013, reviewed in
315 Simmons 2014) but for several reasons female genitalia are still understudied (Ah-King et
316 al. 2014, Brennan and Prum, 2015) even in bushcrickets.
317
318 Amongst species with titillators, shorter sexual refractory periods were associated with more
319 complex titillators. These results appear to be in contrast to the analysis based on the
320 presence/absence of titillators (see above). Shorter sexual refractory periods were associated

with a higher degree of polyandry (this study), so may be used as an indicator of the degree of polyandry. Comparative studies of water-striders (Heteroptera: Gerridae) have similarly found relationships between indices of the degree of polyandry and genital complexity (Rowe and Arnqvist 2012). The results of the present study should be interpreted with caution, however. The relationship between the duration of the female's sexual refractory period and titillator complexity appear to be driven by the two species in the sub-family Zaprochilinae in our dataset, which have unusually long female sexual refractory periods (Simmons and Gwynne 1991; Lehmann and Lehmann 2007) and simple titillators (Rentz, 1993). This subfamily is phylogenetically distinct from the majority of other Tettigoniid families. It should also be noted that we did not find a significant relationship between the actual degree of polyandry itself (rather than the female's sexual refractory period) and titillator complexity in the present study (the two analyses were based on slightly different subsets of species, see Figure 2).

It is possible that the classification scheme of titillators used in the present study does not reflect adequately the characters of the titillators that are subject to sexual selection. An alternative approach would be to use more complex morphometric analyses to quantify the shape and/or to measure the relative sizes of parts of the titillators (see, for example Rowe and Arnqvist 2012). In addition, denser sampling of species within selected titillator-possessing genera would allow for a more fine-scale examination of the relationship between titillator morphology and polyandry. In the present study, most titillator-possessing genera were represented by only three species or fewer.

Although the present study provided mixed support for sexual selection hypotheses for genital evolution, experimental manipulations, in which either one or both titillators were removed, have provided some support for the role of cryptic female choice (Wulff et al. 2015, Vahed 2015, Wulff and Lehmann 2016). The results of these manipulations indicated that correct stimulation by titillators is necessary for the proper insertion of the spermatophore and to avoid resistance by females during copulation (Wulff et al. 2015, 2017; Wulff and Lehmann 2016). Such results, however, are also consistent with the "Lock and Key" hypothesis, a hypothesis that was not tested in the present study. Simmons (2014) pointed out that female choice that enforces species isolation and female choice that targets variation in male quality within populations may be seen as part of the same continuum.

To conclude, the present comparative study provided only partial support for the hypothesis that post-copulatory sexual selection has driven the evolution of titillator complexity in bushcrickets. The inclusion of monandrous species in the sample, examination of the parts of the female's reproductive tract that are contacted by the titillators and denser sampling of selected genera, however, would be necessary to test the sexual selection hypotheses more fully.

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Figure legends

Figure 1: Left hand side: Electron micrograph of Titillator tips with spines in the tettigoniid bushcricket *Metrioptera saussuriana*; Right hand side: Variation in titillator morphology in the Tettigoniidae. The numbers represent the system of titillator classification used in the present study (see Table 1). Images adapted from Rentz and Birchim (1968) and Rentz (1993). Note that titillator types 2 and 3 are shown together because they are similar in overall structure (although the apical part that contacts the female, the median projection, is much less strongly projecting in type 2 than in type 3).

Figure 2: Variation of male and female potential reproductive rate (reciprocal of the sexual refractory period), their ratio (female potential reproductive rate dived by the male potential reproductive rate) and lifetime degree of polyandry, across the bushcricket phylogeny. Data have been scaled such that open and closed circles represent the minimum and maximum in the dataset, respectively. For raw data, see Supplementary Table S1.

Figure 3: a) Lifetime polyandry in bushcricket species lacking titillators ("No") and with titillators ("Yes"); b) Polyandry against titillator complexity (ordinal ranked scale, see Table 1); c) The duration of the male sexual refractory period in bushcricket species lacking titillators ("No") and with titillators ("Yes"); d) The duration of the male sexual refractory period against titillator complexity; e) The duration of the female sexual refractory period in bushcricket species lacking titillators ("No") and with titillators ("Yes"); f) The duration of the female sexual refractory period against titillator complexity; g) Operational sex ratio

559 (measured as the reciprocal of the ratio of the female and male refractory periods) in
560 bushcricket species lacking titillators (“No”) and with titillators (“Yes”); h) Operational sex
561 ratio against titillator complexity.
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563

564 **Table 1**: The titillator classification scheme used in this study (adapted from Vahed et al.

565 2011).

566

Numerical classification	Explanation	Examples
0	Titillators absent	*Poecilimon*
1	No sclerotised titillators, but a densely covered field of small tubercles	*Kawanaphila*
2	One pair of sclerotised titillators, apical part (median projection) conical and not strongly projecting, may have minute teeth	*Ruspolia, Yersinella*
3	One pair of sclerotised titillators: apical part strongly projecting, with no teeth (the tip however can be hooked)	*Metrioptera roeselii*
4	One pair of sclerotised titillators: apical part strongly projecting with clearly visible teeth	*Anonconotus, Decticus*
5	One pair of sclerotised titillators: apical part strongly projecting with teeth concentrated on the club shaped tip	*Metrioptera saussuriana*
6	Two pairs of sclerotised titillators: apical part strongly projecting with teeth on one or both pairs.	*Gampsocleis, Antaxius*

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569 **Supplementary Table S1**: Data base for levels of titillator complexity, polyandry and male

570 and female refractory periods.